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PROVISIONAL APPLICATION FOR PATENT

under

37 CFR §1.53(c)

TITLE: MODIFIED BLOOD CLOTTING FACTORS AND METHODS OF USE

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inhibitors to factors VIII or IX when infused intravenously in amounts sufficient to yield plasma levels of 3-4 μg/mL (60-80 nM).

Factor VII circulates in plasma as a single chain zymogen (M_r = 50,000; 406 amino acids) at a concentration of 0.5 μg/mL (10 nM). It only becomes active following cleavage of a single peptide bond (Arg^{152}-Ile^{153}). The resulting factor VIIa is composed of a light chain (M_r = 20,000) and a heavy chain (M_r = 30,000) associated through a disulfide bond. Since the naturally synthesized factor VII is inactive, using a gene delivery vector carrying factor VII would only increase the absolute circulating level of zymogen. The invention proposes the use of an engineered factor VII such that upon synthesis and secretion from the cell, it is released as active factor VII (FVIIa). In order to accomplish this a protease cleavage site (such as, but not limited to, a PACE/furin site) will be inserted at the normal site of activation (between amino acids Arg^{152}-Ile^{153}). Thus, upon synthesis of the engineered factor VII in the endoplasmic reticulum and Golgi apparatus, the protease recognizing the inserted cleavage site will proteolyze the engineered factor VII releasing a small peptide and generating two chain activated factor VII (FVIIa) which is then released into the circulation.

This invention constitutes a new strategy for treating hemophilia A and B with or without inhibitors using a gene therapy approach with activated factor VII. The invention comprises both a new process and composition of matter.

2. Please list any journal citation, patents, general knowledge or other public information (*prior art*) which describes a similar invention. How does this invention differ from present technology? What problems does it solve or what advantage does it possess?

While the treatment of patients with hemophilia A or B (with inhibitors against factor VIII or factor IX) with recombinant factor VIIa protein has been well-documented and is currently in use, a gene therapy approach using factor VIIa has not been described and would be a novel process.

The present technology for the treatment of hemophilia A or B patients with inhibitors involves infusion with recombinant factor VIIa protein. Because the factor VIIa protein has a very short half-life in the circulation, it must be infused intravenously as frequently as every two hours, increasing the cost and inconvenience of treatment. Our invention would solve this problem by providing continuous endogenous secretion of the active coagulant protein. Additionally, this invention could have uses for other hemostatic disorders such as, but not limited to, Glanzmann's and Bernard-Soulier's thrombasthenia.

In other gene-based approaches to treating hemophiliacs, a major concern is the immune response to the transgene product, which is a protein to which many people with hemophilia are not tolerant. However, all patients with hemophilia A or B should be tolerant to factor VIIa. Therefore, the invention also overcomes a major potential limitation of gene-based approaches to treating hemophilia.

The target tissues for the application of the invention will be, but not limited to, muscle and/or liver. Both tissues have been shown to express transgenes at high levels. For example, injection of an AAV vector with a human factor IX transgene under the control of the elongation factor 1α promoter in the portal vein of mice resulted in levels of 0.7 to 3.3 μg/mL of factor IX protein in the circulation (Nakai, H., et al., Blood 91, 4600-4607, 1998.). These levels of transgene product would be in the therapeutic range for activated factor VII (FVIIa) in a similar gene therapy setting.

3. Describe the invention completely, including, its physical characteristics, operation and the process of making it. Please include all test data proving its utility.

The invention comprises both of a new process and composition of matter. The new process involves the treatment of hemophilia patients (A or B; with or without inhibitory antibodies) or patients presenting with other hemostatic disorders using a gene therapy based approach with activated factor VII. The novel composition of matter constitutes a suitable gene delivery vector carrying an engineered factor VII gene (see Section II, part 1). For a detailed description of the process and composition of matter see accompanying figures and legends.

PRELIMINARY DATA:

An engineered factor VII was created with a protease cleavage site for PACE/furin inserted between amino acids Arg^{152}-Ile^{153} using standard molecular biology techniques. This construct and wild-type human factor VII DNA sequences were inserted into a mammalian expression plasmid.

Human embryonic kidney 293 cells were transiently transfected with either engineered human factor VII, wild-type human factor VII, or no DNA (control). Conditioned media was collected 48 hr post-transfections and was used in a specific factor VII/VIIa clotting assay (prothrombin time clotting assay using factor VII-deficient plasma). This assay reports the time to clot formation which is dependent upon the amount of factor VII or VIIa introduced in the assay. Clot formation will be faster if activated factor VII (FVIIa) is introduced in the assay compared to the zymogen factor VII (wild-type) at the same concentrations.

Results from the clotting time assay indicate that engineered factor VII shortens the clotting time to 18 seconds in contrast to wild-type factor VII which shortened the clotting time to 40 seconds. Similar amounts of factor VII/VIIa protein were introduced into the clotting assay. Control media gave a clotting time of 114 seconds. These results indicate that engineered factor VII is effectively processed to activated factor VII (FVIIa), secreted into the media, and can shorten the prothrombin clotting time to a greater extent than wild-type factor VII.

4. If not included previously, what are possible uses for the invention? In addition to immediate applications, are there other uses that might be realized in the future?

The invention could be used to treat patients with hemophilia A and hemophilia B; patients with hemophilia A and B who have inhibitory antibodies to either factor VIII or factor IX, respectively; and other hemostatic disorders (see Section II, part 2).

Figure Legends:

Figure 1: Engineering Factor VII to Factor VIIa: Pre-pro human factor VII is comprised of 406 amino acids and contains the following domains: signal peptide, propeptide, Gla domain (γ-carboxyglutamic acid domain), EGF-1 (epidermal growth factor domain-1), EGF-2, and the serine protease domain (catalytic domain). The disulfide bond is formed between amino acids Cys^{135} and Cys^{262}. Wild-type human factor VII is secreted as an inactive protein. The engineered human factor VII with a protease cleavage site results in the processing of inactive factor VII to activated factor VII (FVIIa), and is then secreted into the circulation.

Figure 2: Gene Delivery Vector Carrying Engineered Human Factor VII: Engineered human factor VII is inserted into a suitable gene delivery vector, for example adeno-associated virus. The gene delivery vector shown is comprised of the following elements: ITR, inverted terminal repeat; Promoter, with or without tissue specificity or other regulatory sequences such as, but not limited to, enhancers or inducible elements; Engineered human factor VII; Poly A, polyadenylation sequence. Other elements which may enhance expression or processing, for example intronic sequences, may also be included. The vector will be introduced into the target tissue/organ (muscle or liver, but not exclusively) by the most appropriate route of administration.